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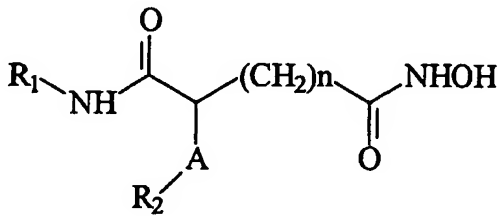
(54) Title: NOVEL CLASS OF CYTODIFFERENTIATING AGENTS AND HISTONE DEACETYLASE INHIBITORS, AND METHODS OF USE THEREOF



(I)

(57) Abstract: The present invention provides the compound having formula (I), wherein each of R_1 and R_2 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, t-butyl, aryloxy, arylalkyloxy, or pyridine group; wherein A is an amido moiety, -O-, -S-, -NH-, or $-CH_2-$; and wherein n is an integer from 3 to 8. The present invention also provides a method of selectively inducing growth arrest, terminal differentiation and/or apoptosis of neoplastic cells and thereby inhibiting proliferation of such cells. Moreover, the present invention provides a method of treating a patient having a tumor characterized by proliferation of neoplastic cells. Lastly, the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically acceptable amount of the compound above.

WO 01/18171 A2

NOVEL CLASS OF CYTODIFFERENTIATING AGENTS AND HISTONE DEACETYLASE INHIBITORS, AND METHODS OF USE THEREOF

This application claims the benefit of U.S. Provisional Application No. 60/208,688, filed June 1, 2000, and U.S. Provisional Application No. 60/152,755, filed September 8, 1999.

Throughout this application various publications are referenced by Arabic numerals within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Background of the Invention

Cancer is a disorder in which a population of cells has become, in varying degrees, unresponsive to the control mechanisms which normally govern proliferation and differentiation. A recent approach to cancer therapy has been to attempt induction of terminal differentiation of the neoplastic cells (1). In cell culture models differentiation has been reported by exposure of cells to a variety of stimuli, including: cyclic AMP and retinoic acid (2,3), aclarubicin and other anthracyclines (4).

There is abundant evidence that neoplastic transformation does not necessarily destroy the potential of cancer cells to differentiate (1,5,6). There are many examples of tumor cells which do not respond to the normal regulators of proliferation and appear to be blocked in the expression of their differentiation program, and yet can be induced to differentiate and cease replicating. A variety of agents, including some relatively simple polar compounds (5,7-9), derivatives of vitamin D and retinoic acid (10-12), steroid hormones (13), growth factors (6,14), proteases (15,16), tumor promoters

(17,18), and inhibitors of DNA or RNA synthesis (4,19-24), can induce various transformed cell lines and primary human tumor explants to express more differentiated characteristics.

Early studies by the some of present inventors identified a series of polar compounds that were effective inducers of differentiation in a number of transformed cell lines (8,9). One such effective inducer was the hybrid polar/apolar compound N,N'-hexamethylene bisacetamide (HMBA) (9), another was suberoylanilide hydroxamic acid (SAHA) (39, 50). The use of these compounds to induce murine erythroleukemia (MEL) cells to undergo erythroid differentiation with suppression of oncogenicity has proved a useful model to study inducer-mediated differentiation of transformed cells (5,7-9).

HMBA-induced MEL cell terminal erythroid differentiation is a multistep process. Upon addition of HMBA to MEL cells (745A-DS19) in culture, there is a latent period of 10 to 12 hours before commitment to terminal differentiation is detected. Commitment is defined as the capacity of cells to express terminal differentiation despite removal of inducer (25). Upon continued exposure to HMBA there is progressive recruitment of cells to differentiate. The present inventors have reported that MEL cell lines made resistant to relatively low levels of vincristine become markedly more sensitive to the inducing action of HMBA and can be induced to differentiate with little or no latent period (26).

HMBA is capable of inducing phenotypic changes consistent with differentiation in a broad variety of cells lines (5). The characteristics of the drug induced effect have been most extensively studied in the murine erythroleukemia cell system (5,25,27,28). MEL cell induction of differentiation is both time and concentration dependent. The minimum concentration required to demonstrate an effect in vitro in most strains is 2 to 3 mM; the minimum duration of continuous exposure generally

required to induce differentiation in a substantial portion (>20%) of the population without continuing drug exposure is about 36 hours.

There is evidence that protein kinase C is involved in the pathway of inducer-mediated differentiation (29). The *in vitro* studies provided a basis for evaluating the potential of HMBA as a cytodifferentiation agent in the treatment of human cancers (30). Several phase I clinical trials with HMBA have been completed (31-36). Clinical trials have shown that this compound can induce a therapeutic response in patients with cancer (35,36). However, these phase I clinical trials also have demonstrated that the potential efficacy of HMBA is limited, in part, by dose-related toxicity which prevents achieving optimal blood levels and by the need for intravenous administration of large quantities of the agent, over prolonged periods. Thus, some of the present inventors have turned to synthesizing compounds that are more potent and possibly less toxic than HMBA (37).

Recently, a class of compounds that induce differentiation, have been shown to inhibit histone deacetylases. Several experimental antitumor compounds, such as trichostatin A (TSA), trapoxin, suberoylanilide hydroxamic acid (SAHA), and phenylbutyrate have been shown to act, at least in part, by inhibiting histone deacetylases (38, 39, 42). Additionally, diallyl sulfide and related molecules (43), oxamflatin, (44), MS-27-275, a synthetic benzamide derivative, (45) butyrate derivatives (46), FR901228 (47), depudecin (48), and m-carboxycinnamic acid bishydroxamide (39) have been shown to inhibit histone deacetylases. In *vitro*, these compounds can inhibit the growth of fibroblast cells by causing cell cycle arrest in the G1 and G2 phases (49-52), and can lead to the terminal differentiation and loss of transforming potential of a variety of transformed cell lines (49-51). In vivo, phenylbutyrate is effective in the treatment of acute

promyelocytic leukemia in conjunction with retinoic acid (53). SAHA is effective in preventing the formation of mammary tumors in rats, and lung tumors in mice (54, 55).

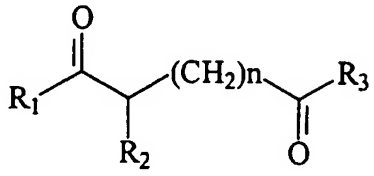
U.S. Patent No. 5,369,108 (41) issued to some of the present inventors discloses compounds useful for selectively inducing terminal differentiation of neoplastic cells, which compounds have two polar end groups separated by a flexible chain of methylene groups, wherein one or both of the polar end groups is a large hydrophobic group. Such compounds are stated to be more active than HMBA and HMBA related compounds.

However, U.S. Patent No. 5,369,108 does not disclose that an additional large hydrophobic group at the same end of the molecule as the first hydrophobic group would further increase differentiation activity about 100 fold in an enzymatic assay and about 50 fold in a cell differentiation assay.

This new class of compounds of the present invention may be useful for selectively inducing terminal differentiation of neoplastic cells and therefore aid in treatment of tumors in patients.

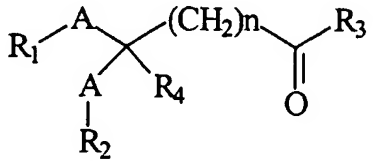
Summary of the Invention

The subject invention provides a compound having the formula:



wherein R_1 and R_2 are the same or different and are each a hydrophobic moiety; wherein R_3 is hydroxamic acid, hydroxylamino, hydroxyl, amino, alkylamino, or alkyloxy group; and n is an integer from 3 to 10, or a pharmaceutically acceptable salt thereof.

The subject invention also provides A compound having the formula:



wherein each of R_1 and R_2 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group; wherein R_3 is hydroxamic acid, hydroxylamino, hydroxyl, amino, alkylamino, or alkyloxy group; wherein R_4 is hydrogen, a halogen, a phenyl, or a cycolalkyl moiety; wherein A may be the same or different and represents an amide moiety, -O-, -S-, $-NR_5-$, or $-CH_2-$, where R_5 is a substituted or unsubstituted C_1-C_5 alkyl; and wherein n is an integer from 3 to 10, or a pharmaceutically acceptable salt thereof.

The subject invention also provides a method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells which comprises contacting the cells under suitable conditions with an effective amount of the aforementioned compound.

Description of the Figures

Figure 1. The effect of Compound 1 according to the subject invention on MEL cell differentiation.

Figure 2. The effect of Compound 1 according to the subject invention on Histone Deacetylase 1 activity.

Figure 3. The effect of Compound 2 according to the subject invention on MEL cell differentiation.

Figure 4. The effect of Compound 3 according to the subject invention on MEL cell differentiation.

Figure 5. The effect of Compound 3 according to the subject invention on Histone Deacetylase 1 activity.

Figure 6. The effect of Compound 4 according to the subject invention on MEL cell differentiation.

Figure 7. The effect of Compound 4 according to the subject invention on Histone Deacetylase 1 activity.

Figure 8. A photoaffinity label (3H-498) binds directly to HDAC 1

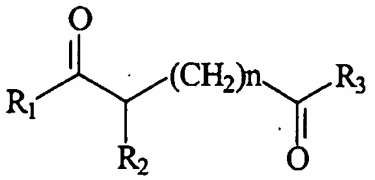
Figure 9. SAHA causes accumulation of acetylated histones H3 and H4 in the CWR22 tumor xenograft in mice.

Figure 10. SAHA causes accumulation of acetylation histones H3 and H4 in peripheral blood monnuclear cells in patients. SAHA was administered by IV infusion daily x 3. Samples were isolated before (Pre), following infusion (Post) and 2 hours after infusion.

Figures 11a-11f. Show the effect of selected compounds on affinity purified human epitope-tagged (Flag) HDAC1.

Detailed Description of the Invention

The subject invention provides a compound having the formula:



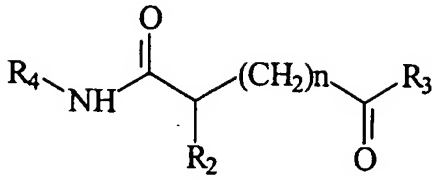
wherein R_1 and R_2 are the same or different and are each a hydrophobic moiety; wherein R_3 is hydroxamic acid, hydroxylamino, hydroxyl, amino, alkylamino, or alkyloxy group; and n is an integer from 3 to 10; or a pharmaceutically acceptable salt of the compound.

In the foregoing compound each of R_1 and R_2 is directly attached or through a linker, and is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group.

Where a linker is used, the linker may be an amide moiety, -O-, -S-, -NH-, or $-CH_2-$.

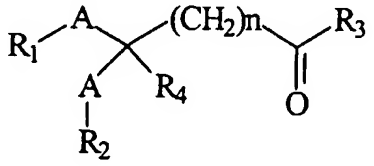
According to this invention, n may be 3-10, preferably 3-8, more preferably 3-7, yet more preferably 4, 5 or 6, and most preferably 5.

In another embodiment of the invention, the compound has the formula:



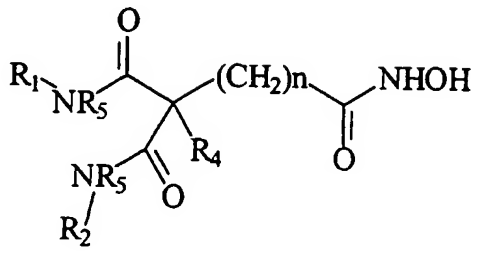
wherein each of R_4 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group. R_2 may be -amide-R_5, wherein R_5 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group.

In a further embodiment of the invention the compound has the formula:

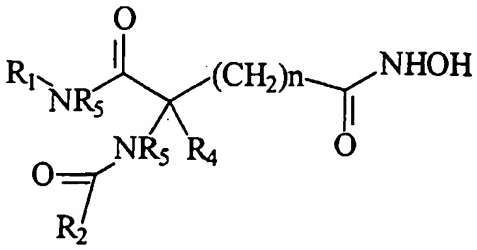


wherein each of R_1 and R_2 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group; wherein R_3 is hydroxamic acid, hydroxylamino, hydroxyl, amino, alkylamino, or alkyloxy group; wherein R_4 is hydrogen, a halogen, a phenyl, or a cycolalkyl moiety; wherein A may be the same or different and represents an amide moiety, -O-, -S-, -NR_5-, or -CH_2-, where R_5 is a substituted or unsubstituted C_1-C_5 alkyl; and wherein n is an integer from 3 to 10, or a pharmaceutically acceptable salt thereof.

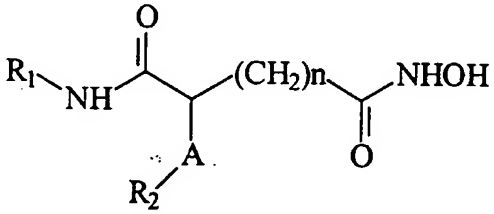
In another embodiment the compound has the formula:



In yet another embodiment, the compound has the formula:



In a further embodiment, the compound has the formula:

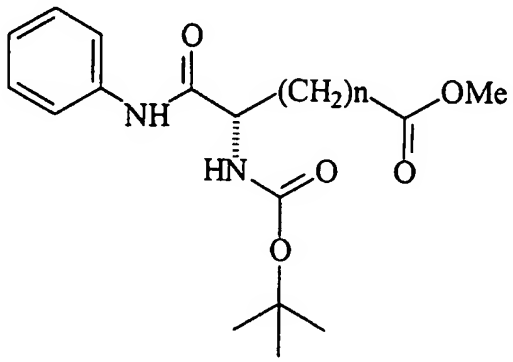


wherein each of R_1 and R_2 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, *t*-butyl, aryloxy, arylalkyloxy, or pyridine group; and wherein n is an integer from 3 to 8.

The aryl or cycloalkyl group may be substituted with a methyl, cyano, nitro, trifluoromethyl, amino, aminocarbonyl, methylcyano, chloro, fluoro, bromo, iodo, 2,3-difluoro, 2,4-difluoro, 2,5-difluoro, 3,4-difluoro, 3,5-difluoro, 2,6-difluoro, 1,2,3-trifluoro, 2,3,6-trifluoro, 2,4,6-trifluoro,

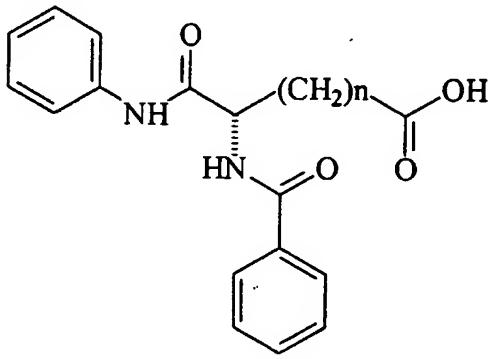
3,4,5-trifluoro, 2,3,5,6-tetrafluoro, 2,3,4,5,6-pentafluoro, azido, hexyl, t-butyl, phenyl, carboxyl, hydroxyl, methoxy, phenyloxy, benzyloxy, phenylaminooxy, phenylaminocarbonyl, methyoxycarbonyl, methylaminocarbonyl, dimethylamino, dimethylaminocarbonyl, or hydroxylaminocarbonyl group.

In a further embodiment, the compound has the formula:



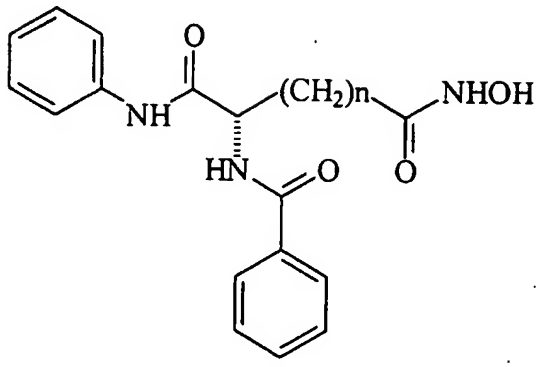
or an enantiomer thereof.

In a yet further embodiment, the compound has the formula:



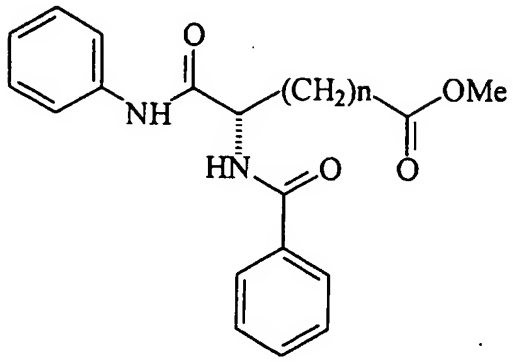
or an enantiomer thereof.

In a further embodiment, the compound has the formula:



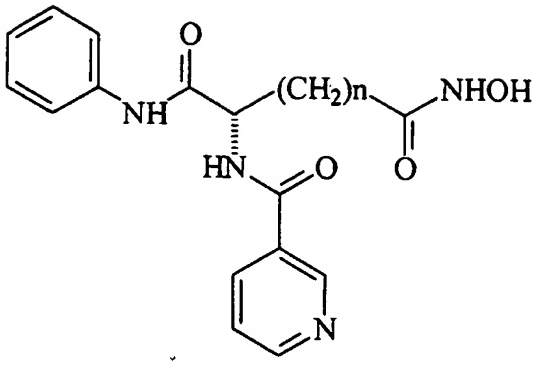
or an enantiomer thereof.

In a yet further embodiment, the compound has the formula:



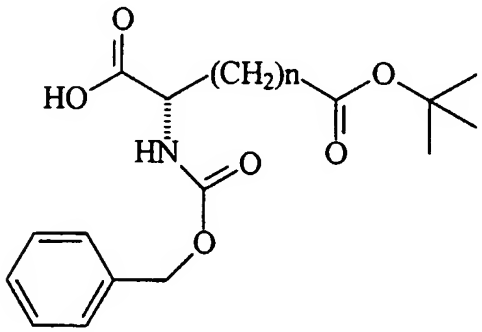
or an enantiomer thereof.

In a further embodiment, the compound has the formula:



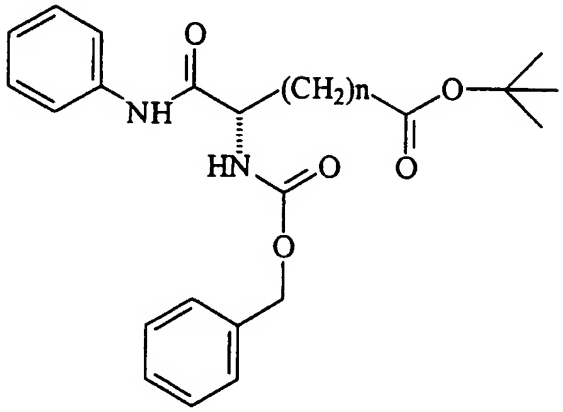
or an enantiomer thereof.

In a yet further embodiment, the compound has the formula:



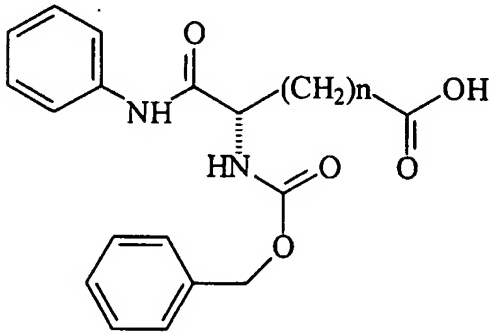
or an enantiomer thereof.

In a yet further embodiment, the compound has the formula:



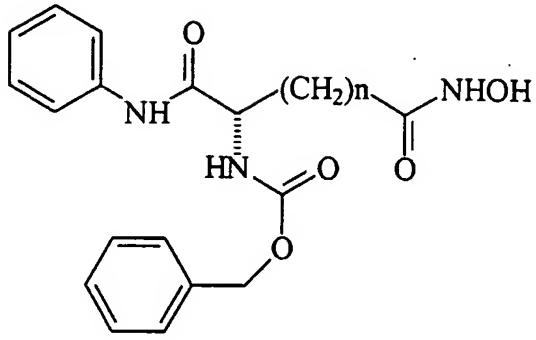
or an enantiomer thereof.

In a further embodiment, the compound has the formula:



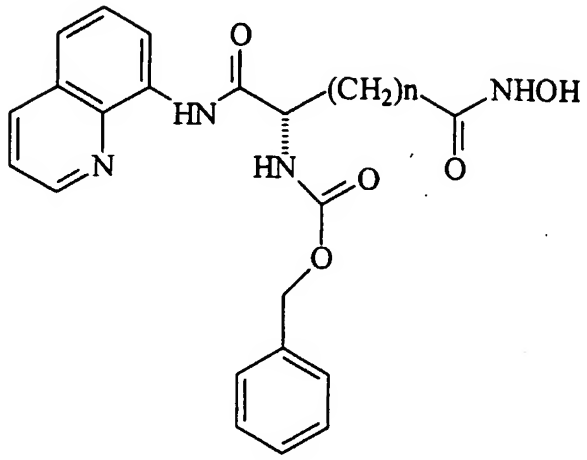
or an enantiomer thereof.

In a further embodiment, the compound has the formula:



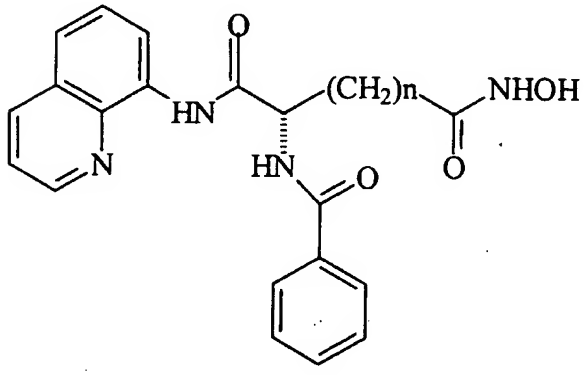
or an enantiomer thereof.

In a yet further embodiment, the compound has the formula:



or an enantiomer thereof.

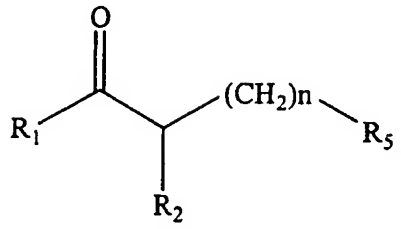
In a further embodiment, the compound has the formula:



or an enantiomer thereof.

This invention is also intended to encompass enantiomers and salts of the compounds listed above.

In a further embodiment, the compound has the formula:



wherein R_1 and R_2 are the same or different and are each a hydrophobic moiety;

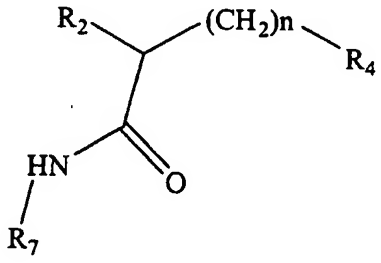
wherein R_5 is $-C(O)-NHOH$ (hydroxamic acid), $-C(O)-CF_3$ (trifluoroacetyl), $-NH-P(O)OH-CH_3$, $-SO_2NH_2$ (sulfonamide), $-SH$ (thiol), $-C(O)-R_6$, wherein R_6 is hydroxyl, amino, alkylamino, or alkyloxy group; and

n is an integer from 3 to 10, or a pharmaceutically acceptable salt thereof.

In the foregoing compund, each of R_1 and R_2 may be directly attached or through a linker, and is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group.

The linker may be an amide moiety, -O-, -S-, -NH-, or $-CH_2-$.

In another embodiment, the compound has the formula:

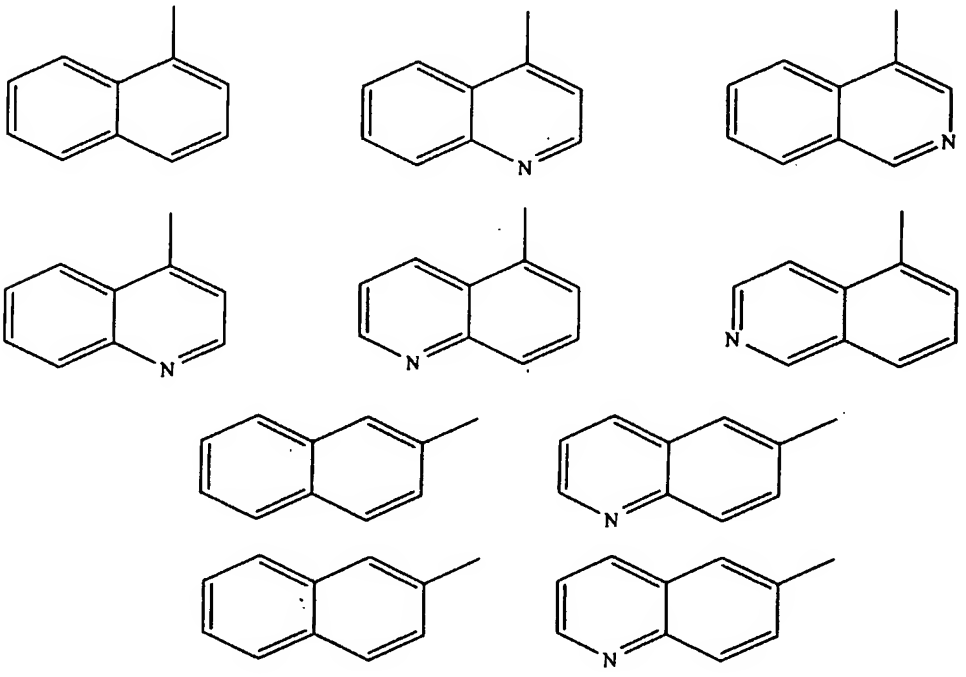


wherein each of R_7 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino,

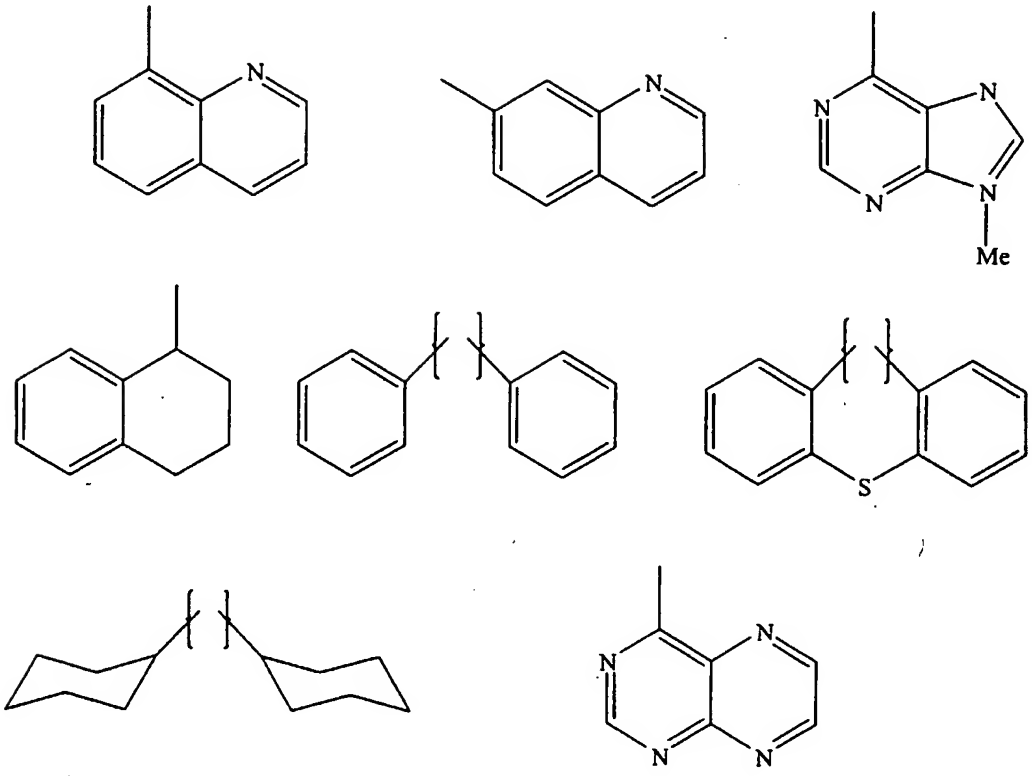
9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group.

In the foregoing compound, R_2 may be -sulfonamide-R_8, or -amide-R_8, wherein R_8 is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group.

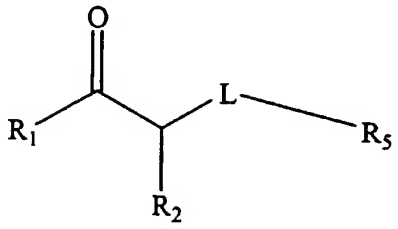
The R_2 may be -NH-C(O)-Y, -NH-SO_2-Y, wherein Y is selected from the group consisting of:



The R_7 may be selected from the group consisting of:



In yet another embodiment, the compound has the formula:



wherein R_1 and R_2 are the same or different and are each a hydrophobic moiety;

wherein R_5 is $-C(O)-NHOH$ (hydroxamic acid), $-C(O)-CF_3$ (trifluoroacetyl), $-NH-P(O)OH-CH_3$, $-SO_2NH_2$ (sulfonamide), -SH (thiol), $-C(O)-R_6$, wherein R_6 is hydroxyl, amino, alkylamino, or alkyloxy group; and

wherein L is a linker consisting of $-(CH_2)-$, $-C(O)-$, -S-, -O-, $-(CH=CH)-$, -phenyl-, or -cycloalkyl-, or any combination thereof,

or a pharmaceutically acceptable salt thereof.

L may also be a linker consisting of $-(CH_2)_n-$, $-C(O)-$, -S-, -O-, $-(CH=CH)_m-$, -phenyl-, or -cycloalkyl-, or any combination thereof, wherein n is an integer from 3 to 10, and m is an integer from 0 to 10,

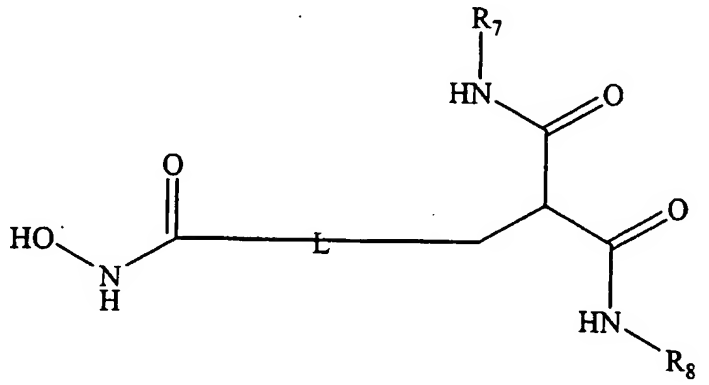
In the foregoing compound, n may be from 4-7, and m is from 0-7. Preferably n is 5 or 6, most preferably n is 6. Preferably m is from 1-6, more preferably m is 2-5, most preferably m is 3 or 4,

In the compound, each of R_1 and R_2 may be directly attached or through a linker, and is, substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group.

The linker may be an amide moiety, -O-, -S-, -NH-, or $-CH_2-$.

This invention is also intended to encompass enantiomers, salts and pro-drugs of the compounds disclosed herein.

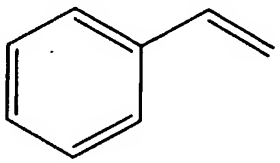
In another embodiment the compound may have the formula:



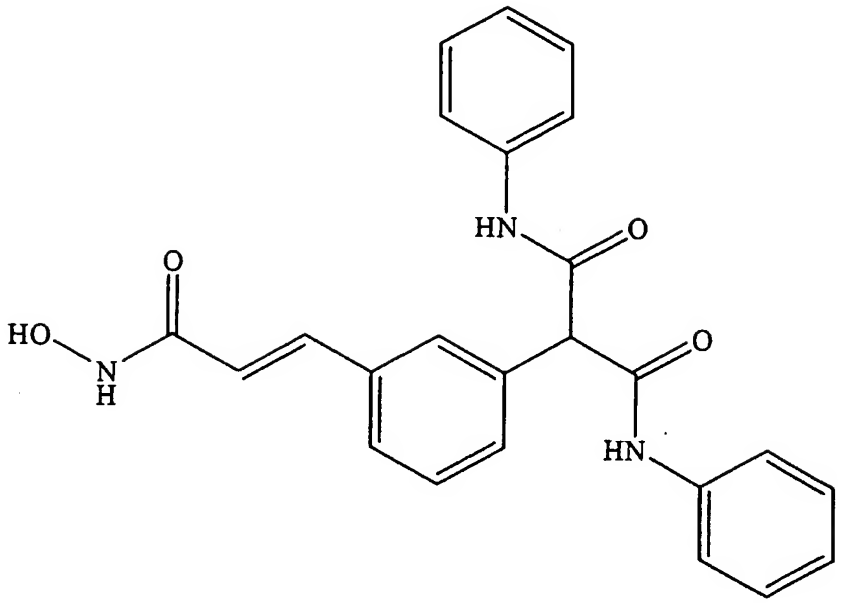
wherein L is a linker selected from the group consisting of -(CH_2)-, -(CH=CH)-, -phenyl-, -cycloalkyl-, or any combination thereof; and

wherein each of R_7 and R_8 are independently substituted or unsubstituted, aryl, cycloalkyl, cycloalkylamino, naphtha, pyridineamino, piperidino, 9-purine-6-amine, thiazoleamino group, hydroxyl, branched or unbranched alkyl, alkenyl, alkyloxy, aryloxy, arylalkyloxy, or pyridine group.

In a preferred embodiment, the linker L comprises the moiety



In another preferred embodiment, the compound has the formula:



Any of the disclosed compounds can be formed into a pharmaceutical composition together with a pharmaceutically acceptable carrier.

Any of the compounds can also be formed into a pharmaceutically acceptable salt of the compound using well known pharmacological techniques.

A prodrug of any of the compounds can also be made using well known pharmacological techniques.

Any of the compounds can be used in a method of inducing differentiation of tumor cells in a tumor comprising contacting the cells with an effective amount of the compound so as to thereby differentiate the tumor cells.

Any of the compounds can also be used in a method of inhibiting the activity of histone deacetylase comprising contacting the histone deacetylase with an effective amount of the compound so as to thereby inhibit the activity of histone deacetylase.

This invention, in addition to the above listed compounds, is further intended to encompass the use of homologs and analogs of such compounds. In this context, homologs are molecules having substantial structural similarities to the above-described compounds and analogs are molecules having substantial biological similarities regardless of structural similarities.

In a further embodiment, the subject invention provides a pharmaceutical composition comprising a pharmaceutically effective amount of any one of the aforementioned compounds and a pharmaceutically acceptable carrier.

In a yet further embodiment, the subject invention provides a method of selectively inducing growth arest, terminal differentiation and/or apoptosis of neoplastic cells and thereby inhibiting proliferation of such cells which comprises contacting the cells under suitable conditions with an effective amount of any one of the aforementioned compounds.

The contacting should be performed continuously for a prolonged period of time, i.e. for at least 48 hours, preferably for about 4-5 days or longer.

The method may be practiced *in vivo* or *in vitro*. If the method is practiced in vitro, contacting may be effected by incubating the cells with the compound. The concentration of the compound in contact with the cells should be from about 1 nM to about 25 mM, preferably from about 20 nM to about 25 mM, more preferably from about 40 nM to 100 μM, yet more preferably from about 40 nM to about 200 nM. The concentration depends upon the individual compound and the state of the neoplastic cells.

The method may also comprise initially treating the cells with an antitumor agent so as to render them resistant to an antitumor agent and subsequently contacting the resulting

resistant cells under suitable conditions with an effective amount of any of the compounds above, effective to selectively induce terminal differentiation of such cells.

The present invention also provides a method of treating a patient having a tumor characterized by proliferation of neoplastic cells which comprises administering to the patient an effective amount of any of the compounds above, effective to selectively induce growth arrest, terminal differentiation and/or apoptosis of such neoplastic cells and thereby inhibit their proliferation.

The method of the present invention is intended for the treatment of human patients with tumors. However, it is also likely that the method would be effective in the treatment of tumors in other mammals. The term tumor is intended to include any cancer caused by the proliferation of neoplastic cells, such as prostate cancer, lung cancer, acute leukemia, multiple myeloma, bladder carcinoma, renal carcinoma, breast carcinoma, colorectal carcinoma, neuroblastoma or melanoma.

Routes of administration for the compound of the present invention include any conventional and physiologically acceptable route, such as, for example, oral, pulmonary, parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), inhalation (via a fine powder formulation or a fine mist), transdermal, nasal, vaginal, rectal, or sublingual routes of administration and can be formulated in dosage forms appropriate for each route of administration.

The present invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier, such as sterile pyrogen-free water, and a therapeutically acceptable amount of any of the compounds above. Preferably, the effective amount is an amount effective to selectively induce terminal

differentiation of suitable neoplastic cells and less than an amount which causes toxicity in a patient.

The present invention provides the pharmaceutical composition above in combination with an antitumor agent, a hormone, a steroid, or a retinoid.

The antitumor agent may be one of numerous chemotherapy agents such as an alkylating agent, an antimetabolite, a hormonal agent, an antibiotic, colchicine, a vinca alkaloid, L-asparaginase, procarbazine, hydroxyurea, mitotane, nitrosoureas or an imidazole carboxamide. Suitable agents are those agents which promote depolarization of tubulin. Preferably the antitumor agent is colchicine or a vinca alkaloid; especially preferred are vinblastine and vincristine. In embodiments where the antitumor agent is vincristine, an amount is administered to render the cells are resistant to vincristine at a concentration of about 5 mg/ml. The administration of the agent is performed essentially as described above for the administration of any of the compounds. Preferably, the administration of the agent is for a period of at least 3-5 days. The administration of any of the compounds above is performed as described previously.

The pharmaceutical composition may be administered daily in 2-6 hour infusions for a period of 3-21 days, for example, daily in a 4 hour infusion for a period of 5 days.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

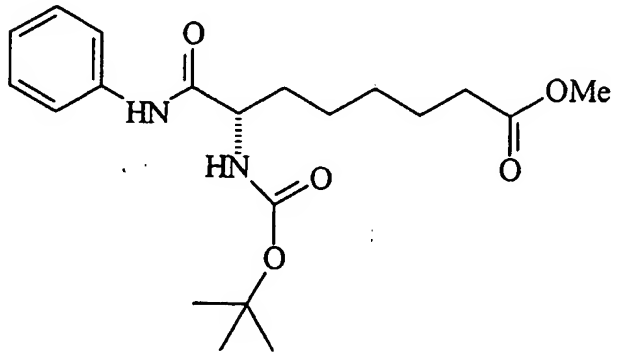
Examples 1-5 show the synthesis of substituted L-α-aminosuberic hydroxamic acids according to the subject invention, and Examples 6 and 7 show the effects of compounds 1-5 on MEL cell differentiation and Histone Deacetylase activity.

Example 1 - Synthesis of Compound 1

N-Boc-ω-methyl-(L)-α-aminosuberate, Boc-Asu(OMe) was prepared according to a published procedure (40). ("Boc" = t-butoxycarbonyl; "Asu" = α-aminosuberate (or α-aminosuberic acid))

N-Cbz-ω-t-butyl-(L)-α-aminosuberate, dicyclohexylamine salt was purchased from Research Plus, Bayonne, NJ.

N-Boc-ω-methyl-(L)-α-aminosuberateanilide, Boc-Asu(OMe)-NHPh.



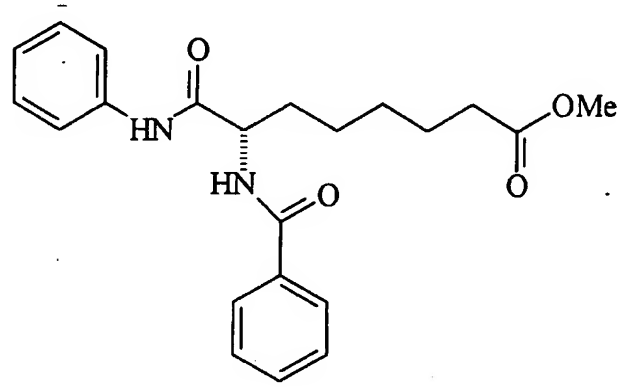
(I1)

N-Boc-ω-methyl-(L)-α-aminosuberate (493mg, 1.63mmoles) was dissolved under Ar in 7mL of dry CH_2Cl_2. EDC (470mg, 2.45mmoles) was added, followed by aniline (230μL, 2.52 mmoles). The solution was stirred at room temperature for 2h 30min, then washed with dilute HCl (pH 2.4, 2x5mL), sat. $NaHCO_3$ (10mL), and H_2O (2x10mL). The product was purified by column chromatography

(Silica gel, Hexanes: AcOEt 3.5:1). The isolated yield was 366mg (60%).

^{1}H-NMR and Mass Spectroscopy were consistent with the product.

N-Benzoyl-ω-methyl-(L)-α-aminosuberateanilide, PhCOHN-Asu(OMe)-NHPh.



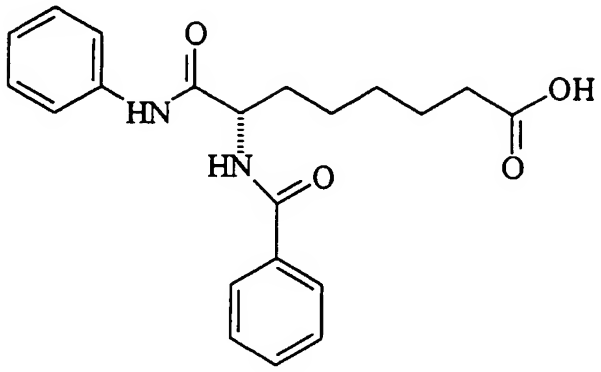
(I2)

90mg of N-Bloc-ω-methyl-(L)-α-aminosuberateanilide (0.238mmoles) were treated with 3.2mL of 25% trifluoroacetic acid (TFA) CH_2Cl_2 for 30 min. The solvent was removed and the residue left under high vacuum for 12h. It was dissolved under Ar in 3mL of dry CH_2Cl_2 and benzotriazole-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP) (149mg, 0.286mmoles), benzoic acid (44mg, 0.357mmoles) and diisopropylethylamine (114μL, 0.655mmoles). The solution was stirred at room temperature for 1h. The product was purified by column chromatography (Silica gel, Hexanes: AcOEt 3:1-2:1) as a white solid: 75mg, 82%.

^{1}H-NMR and Mass Spectroscopy were consistent with the product.

The foregoing coupling reaction was also successfully accomplished using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) as a reagent.

N-Benzoyl-(L)-α-aminosuberoylanilide, PhCONH-Asu(OH)-NHPh.

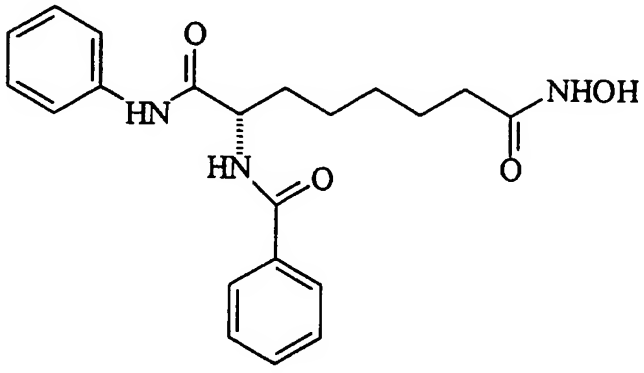


(I3)

75mg (0.196mmoles) of N-benzoyl--aminosuberateanilide were stirred for 6h at 0°C in 1M NaOH:THF:MeOH 1:1:1. After complete disappearance of the starting material, the solution was neutralized (1M HCl) and extracted with AcOEt. The organic phase was collected and dried. Solvent removal yielded the product as a white solid: 67mg, 93%.

^{1}H-NMR and Mass Spectroscopy were consistent with the product.

N-Benzoyl-(L)-α-aminosuberoylanilide-ω-hydroxamic acid, PhCONH-Asu(NHOH)-NHPh:



(1)

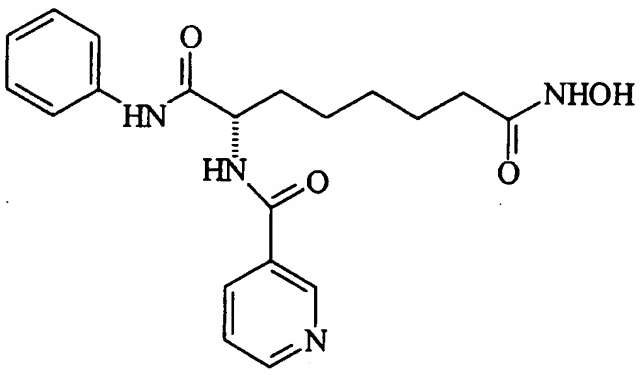
To a suspension of 26mg of N-benzoyl-ω-methyl-(L)-α-aminosuberateanilide (I2) in 1mL of dry CH_2Cl_2 was added 58mg of H_2NOTBDPS (H_2NO-t-butyldiphenylsilyl) followed by 22mg of EDC. The reaction was stirred at room temperature for 4h. The intermediate protected hydroxamic acid was purified by column chromatography (silica gel, CH_2Cl_2: MeOH 100:0-98-2). It was deprotected by treatment with 5% TFA in CH_2Cl_2 for 1h30min. The product was precipitated from acetone-pentane.

^{1}H-NMR (d_6-DMSO, 500MHz) δ= 10.29 (s, 1H), 8.53 (d, 1H), 7.90 (d, 2H), 7.60 (d, 2H), 7.53 (m, 1H), 7.46 (t, 2H), 7.28 (t, 2H), 7.03 (t, 2H), 4.53 (q, 1H), 1.92 (t, 2H), 1.78 (m, 2H), 1.50-1.25 (m, 6H).

ESI-MS : 384 (M+1), 406 (M+Na), 422 (M+K)

Example 2 - Synthesis of Compound 2

N-Nicotinoyl-(L)-α-aminosuberoylanilide-ω-hydroxamic acid, C_5H_4NCO-Asu(NHOH)-NHPh:



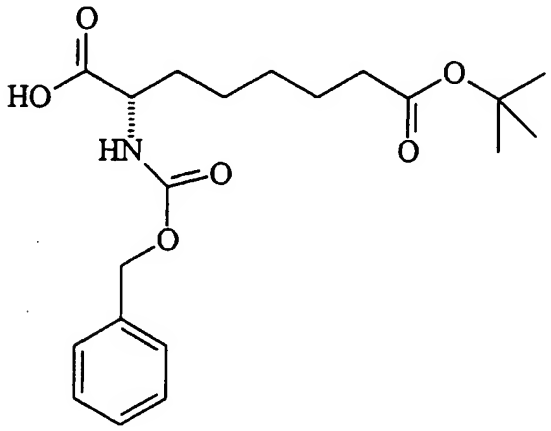
(2)

It was prepared from N-Boc-ω-methyl-L-α-aminosuberate following the same procedure used for the benzoyl analog. Yields and chromatographic behaviour were comparable.

^{1}H-NMR (d_6-DMSO, 500MHz) δ= 10.30 (s, 1H), 10.10 (s, 1H), 9.05 (m, 1H), 8.80 (m, 1H), 8.71 (m, 1H), 8.24 (m, 1H), 7.60 (m, 2H), 7.30 (m, 2H), 7.04 (m, 1H), 4.56 (m, 1H), 1.93 (t, 2H), 1.79 (m, 2H), 1.55-1.30 (m, 6H). ESI-MS : 385 (M+1), 407 (M+Na).

Example 3 - Synthesis of Compound 3

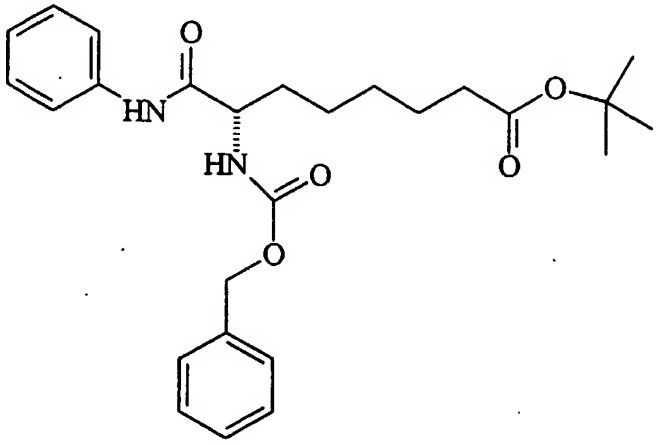
N-benzyloxycarbonyl-ω-*t*-butyl-(L)-aminosuberic acid, N-Cbz-(L)-Asu(O*t*Bu)-OH.



(I4)

N-Cbz-(L)-Asu(O*t*Bu)-OH, dicyclohexylamine salt (100 mg, 0.178 mmol) was partitioned between 1 M HCl (5mL) and EtOAc (10mL). The organic layer was removed, and the aqueous portion washed with EtOAc (3 x 3 mL). The organic fractions were combined, washed with brine (1 x 2 mL), and dried ($MgSO_4$). The mixture was filtered and concentrated to a colorless film (67 mg, 0.176 mmol, 99%). This compound was used immediately in the next step.

N-benzyloxycarbonyl-ω-*t*-butyl-(L)-α-aminosuberateanilide, N-Cbz-(L)-Asu(O*t*Bu)-NHPh.

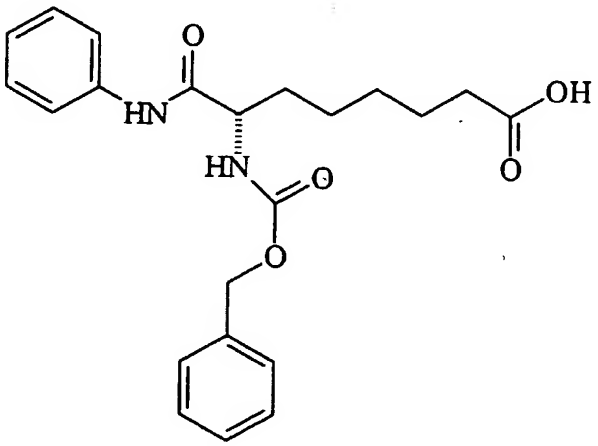


(I5)

N-Cbz-(L)-Asu(OtBu)-OH (67mg, 0.176 mmol) was dissolved in dry CH_2Cl_2 (2.5 mL). Aniline (17 μL, 0.187 mmol), PyBOP (97 mg, 0.187 mmol), and iPr_2NEt (46 μL, 0.266 mmol) were added and the mixture stirred for 2 h. The reaction was complete as indicated by TLC. The mixture was diluted with EtOAc (5 mL) and water (5 mL), and the layers separated. The aqueous portion was washed with EtOAc (3 x 3 mL) and the organic fractions combined. This solution was washed with 1 M HCl (1 x 2 mL) and brine (1 x 2 mL), dried ($MgSO_4$), filtered, and concentrated to a crude oil. This was passed through a plug of silica gel (30% EtOAc/hexanes) to remove baseline impurities, affording the compound (76mg, 0.167 mmol, 94%).

^{1}H NMR ($CDCl_3$, 400 MHz, no TMS) δ 8.20 (br s, 1H), 7.47 (d, 2H), 7.32 (m, 5H), 7.28 (t, 2H), 7.08 (t, 1H), 5.39 (d, 1H), 5.10 (m, 2H), 4.26 (m, 1H), 2,18 (t, 2H), 1.93 (m, 1H), 1.67 (m, 1H), 1.55 (m, 3H), 1.42 (s, 9H), 1.36 (m, 3H).

N-benzyloxycarbonyl-(L)-α-aminosuberateanilide, N-Cbz-(L)-Asu(OH)-NHPh.

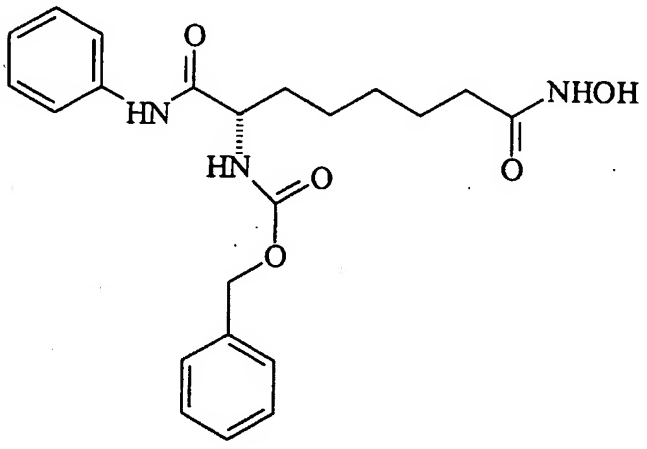


(I6)

N-Cbz-(L)-Asu(O*t*Bu)-anilide (76mg, 0.167 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and TFA (0.5 mL) added dropwise. The reation was complete by TLC after 3h. The mixture was concentrated in vacuo to give the title compound (80 mg, crude). This compound was taken on without purification to the next step.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.93 (br s, 1H), 9.99 (br s, 1H), 7.57 (m, 3H), 7.34 (m, 5H), 7.29 (t, 2H), 7.03 (t, 1H), 5.02 (m, 2H), 4.11 (m, 1H), 2.17 (t, 2H), 1.61 (m, 2H), 1.46 (m, 2H), 1.27 (m, 4H).

N-benzyloxycarbonyl-(L)-α-aminosuberateanilide ω-hydroxamic acid, N-Cbz-(L)-Asu(NH-OH)-NHPh.



(3)

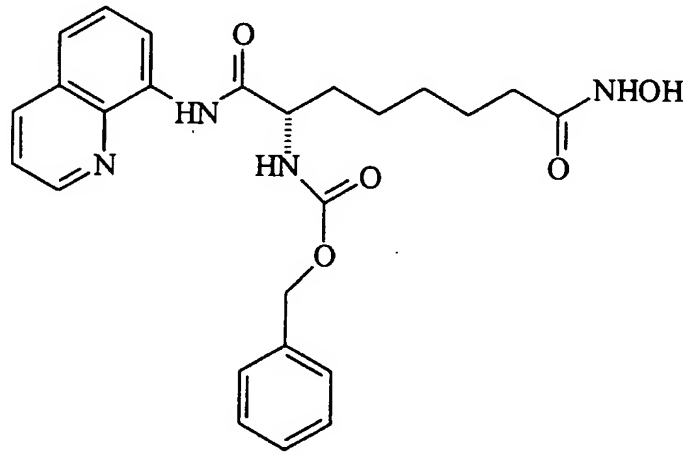
N-Cbz-(L)-Asu(OH)-anilide (80 mg, crude) and O-t-butyldiphenylsilyl-hydroxylamine (60 mg, 0.221 mmol) were dissolved in CH_2Cl_2 (4 mL). To this was added PyBOP (125 mg, 0.241 mmol) and iPr_2NEt (52 μL, 0.302 mmol) and stirred overnight. TLC indicated reaction completion. The mixture was concentrated in vacuo and then passed through a plug of silica gel (50% EtOAc/hexanes) to remove baseline impurities. Evaporation of volatiles afforded 107 mg of material which was then dissolved in dry CH_2Cl_2 (5mL) and TFA (0.25 mL) was added. Monitoring by TLC indicated completion after 1.5h. Concentrated

in vacuo to remove all volatiles. The reside was taken up in EtOAc (3mL), and then hexanes was added slowly to result in the precipitation of a white gel. The supernatant was removed, and the precipitate washed with hexanes (3 x 2 mL). This material was taken to dryness under reduced pressure, to afford the title compound (40 mg, 0.097 mmol, 59%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.32 (s, 1H), 10.00 (s, 1H), 8.64 (br s, 1H), 7.57 (m, 3H), 7.37 (m, 5H), 7.30 (t, 2H), 7.04 (t, 1H), 5.02 (m, 2H), 4.12 (m, 1H), 1.93 (t, 2H), 1.62 (m, 2H), 1.45 (m, 2H), 1.29 (m, 4H); ESI-MS 414 (M+1).

Example 4 - Synthesis of Compound 4

N-benzyloxycarbonyl-(L)-α-aminoxuberoyl-8-quinolinamide-ω-hydroxamic acid.



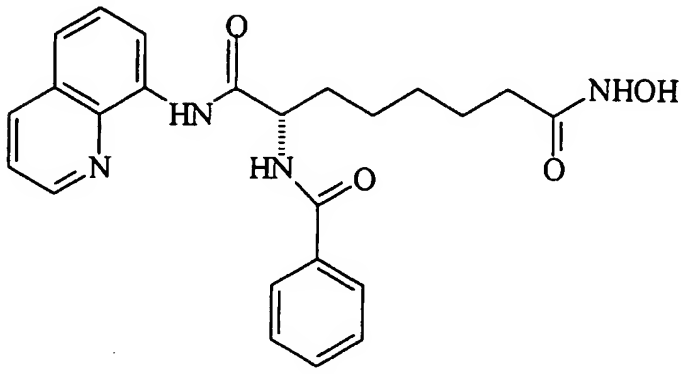
(4)

Prepared in similar manner to compound 3.

1H NMR (DMSO-d6, 400 MHz) δ 10.45 (s, 1H), 10.31 (s, 1H), 8.85 (dd, 1H), 8.63 (dd, 1H), 8.42 (dd, 1H), 8.13 (dd, 1H), 8.68 (m, 2H), 7.60 (t, 1H), 7.37 (m, 2H), 7.28 (m, 2H), 5.10 (m, 2H), 4.24 (m, 1H), 1.93 (t, 2H), 1.85 (m, 1H), 1.70 (m, 1H), 1.50 (m, 2H), 1.42 (m, 2H), 1.30 (m, 2H); ESI-MS 465 (M+1).

Example 5 - Synthesis of Compound 5

N-Benzoyl-(L)-α-aminosuberoyl-8-quinolinamide-ω-hydroxamic acid:

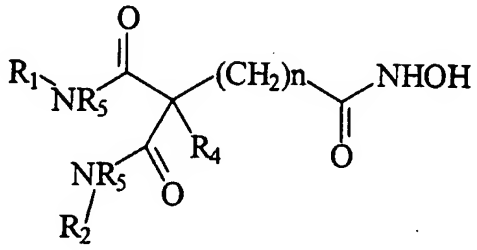


(5)

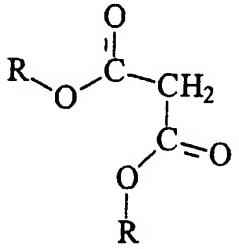
A sample of the N-Cbz-ω-t-butyl L-α-aminosuberoyl-8-quinolinamide (90mg, 0.178 mmoles) was obtained from the previous synthesis. The Cbz group was removed by hydrogenation in MeOH on 5%Pd on C. The resulting free amine was coupled with benzoic acid using EDC in dry CH_2Cl_2 (69% over the two steps). After TFA deprotection of the t-butyl ester, the usual coupling with H_2NOTBDPS followed by deprotection afforded the desired hydroxamic acid.

^{1}H-NMR (d_6-DMSO, 500MHz) δ=10.55 (s, 1H), 10.30 (s, 1H), 9.03 (m, 1H), 8.78 (m, 1H), 8.62 (m, 1H), 8.40 (m, 1HO, 7.97 (m, 2H), 7.67-7.46 (m, 6H), 4.66 (m, 1H), 1.94 (t, 2H), 1.87 (m, 1H), 1.80-1.20 (m, 7H). ESI-MS : 435 (M+1).

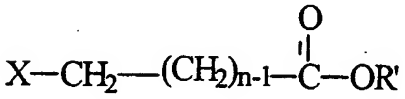
Example 6 - Synthesis of compound with inverted amide group. A compound having the following formula:



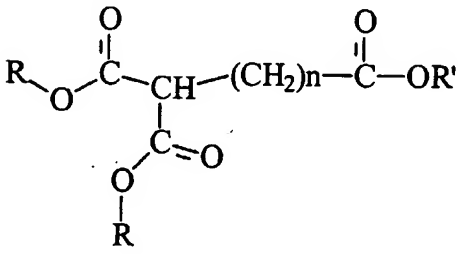
is synthesized by treating a malonic ester:



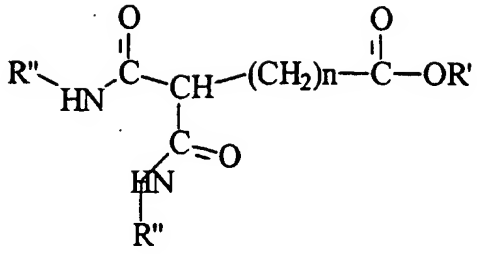
with a base, and then adding:



where X is a halogen, to form:



from which R is removed by reaction with an amine and a carbodiimide reagent ro form:



from which R' is removed and converted to hydroxamic acid (NHOH) as in the previous examples.

In the foregoing scheme, R may be t-butyl, removed with trifluoroacetic acid; R' may be methyl, removed with a base or LiI; and each R'' may be the same or different, depending on the reagent used.

Example 7 - Effect of Compound 1 (N-Benzoyl-(L)-α-aminosuberoylanilide-ω-hydroxamic acid, PhCONH-Asu(NHOH)-NHPh) on MEL Cell Differentiation and Histone Deacetylase Activity

Murine erythroleukemia (MEL) cell differentiation.

The MEL cell differentiation assay was used to assess the ability of Compound 1 to induce terminal differentiation. MEL cells (logarithmically dividing) were cultured with the indicated concentrations of Compound 1. Following a 5-day culture period, cell growth was determined using a Coulter Counter and differentiation was determined microscopically using the benzidine assay to determine hemoglobin protein accumulation on a per cell basis.

It was observed, as shown in Figure 1, that Compound 1 (200nM) is able to induce MEL cell differentiation.

Histone Deacetylase (HDAC) enzymatic activity.

The effect of Compound 1 on affinity purified human epitope-tagged (Flag) HDAC1 was assayed by incubating the enzyme preparation in the absence of substrate on ice for 20 min with

the indicated amounts of Compound 1. Substrate ([^{3}H]acetyl-labeled murine erythroleukemia cell-derived histone) was added and the samples were incubated for 20 min at 37°C in a total volume of 30 µl. The reactions were then stopped and released acetate was extracted and the amount of radioactivity released determined by scintillation counting.

It was observed, as shown in Figure 2, that Compound 1 is a potent inhibitor of HDAC1 enzymatic activity (ID_{50}=1nM).

Example 8 - Effect of Compound 2 (N-Nicotinoyl-(L)-α-aminosuberoylanilide-ω-hydroxamic acid, C_5H_4NCO-Asu(NHOH)-NHPh) on MEL Cell Differentiation

Murine erythroleukemia (MEL) cell differentiation:
The MEL cell differentiation assay was used to assess the ability of Compound 2 to induce terminal differentiation. MEL cells (logarithmically dividing) were cultured with the indicated concentrations of Compound 2. Following a 5-day culture period differentiation was determined microscopically using the benzidine assay to determine hemoglobin protein accumulation on a per cell basis.

It was observed, as shown in Figure 3, that Compound 2 (800nM) is able to induce MEL cell differentiation.

Example 9 - Effect of Compound 3 (N-benzyloxycarbonyl-(L)-α-aminosuberateanilide ω-hydroxamic acid, N-Cbz-(L)-Asu(NH-OH)-NHPh) on MEL Cell Differentiation and Histone Deacetylase Activity

Murine erythroleukemia (MEL) cell differentiation:
The MEL cell differentiation assay was used to assess the ability of Compound 3 to induce terminal differentiation. MEL cells (logarithmically dividing) were cultured with the indicated concentrations of Compound 3. Following a 5-day culture period differentiation was determined microscopically using the benzidine assay to determine hemoglobin protein accumulation on a per cell basis.

It was observed, as shown in Figure 4, that Compound 3 (400nM) is able to induce MEL cell differentiation.

Histone deacetylase (HDAC) enzymatic activity:

The effect of Compound 3 on affinity purified human epitopetagged (Flag) HDAC1 was assayed by incubating the enzyme preparation in the absence of substrate on ice for 20 min with the indicated amounts of HPC. Substrate ([^{3}H]accetyl-labelled murine erythroleukemia cell-derived histone) was added and the samples were incubated for 20 min at 37°C in a total volume of 30 μl. The reactions were then stopped and relaesed acetate was extracted and the amount of radioactivity released determined by scintillation counting.

It was observed, as shown in Figure 5, that Compound 3 is a potent inhibitor of HDAC1 enzymatic activity (ID_{50}-100 nM).

Example 10 - Effect of Compound 4 (N-benzyloxycarbonyl-(L)-α-aminoxuberoyl-8-quinolinamide-ω-hydroxamic acid) on MEL Cell Differentiation and Histone Deacetylase Activity

Murine erythroleukemia (MEL) cell differentiation:

The MEL cell differentiation assay was used to assess the ability of Compound 4 to induce terminal differentiation. MEL cells (logarithmically dividing) were cultured with the indicated concentrations of Compound 4. Following a 5-day culture period differentiation was determined microscopically using the benzidine assay to determine hemoglobin protein accumulaiton on a per cell basis.

It was observed, as shown in Figure 6, that Compound 4 (40 nM) is able to induce MEL cell differentiation.

Histone deacetylase (HDAC) enzymatic activity:

The effect of Compound 4 on affinity purified human epitope-tagged (Flag) HDAC1 was assayed by incubating the enzyme preparation in the absence of substrate on ice for 20 min with indicated amounts of HPC. Substrate ([^{3}H]acetyl-labelled murine

erythroleukemia cell-derived histone) was added and the samples were incubated for 20 min at 37°C in a total volume of 30 μl. The reactions were then stopped and released acetate was extracted and the amount of radioactivity released determined by scintillation counting.

It was observed, as shown in Figure 7, that Compound 4 is a potent inhibitor of HDAC1 enzymatic activity (ID_{50}<10 nM).

SAHA inhibits the activity of affinity purified HDAC1 and HDAC3 (39). Crystallographic studies with SAHA and a HDAC related protein reveal that SAHA inhibits HDAC by a direct interaction with the catalytic site (66). Additional studies demonstrate that a tritium labeled photoaffinity SAHA analog (^{3}H-498) that contains an azide moiety (67) binds directly to HDAC1 (Fig. 8). These results indicate that this class of hydroxamic acid based compound inhibits HDAC activity through a direct interaction with the HDAC protein.

SAHA causes the accumulation of acetylated histones H3 and H4 *in vivo*. The *in vivo* effect of SAHA has been studied using the CWR22 human prostate xenograft in mice (68). SAHA (50 mg/kg/day) caused a 97% reduction in mean final tumor volume compared to controls with no apparent toxicity. SAHA administration at this dose caused an increase in acetylated histones H3 and H4 in the tumor xenograft (Fig 9).

SAHA is currently in Phase I Clinical Trials in patients with solid tumors. SAHA causes an accumulation of acetylated histones H3 and H4 in the peripheral blood mononuclear cells isolated from patients undergoing treatment (Fig. 10).

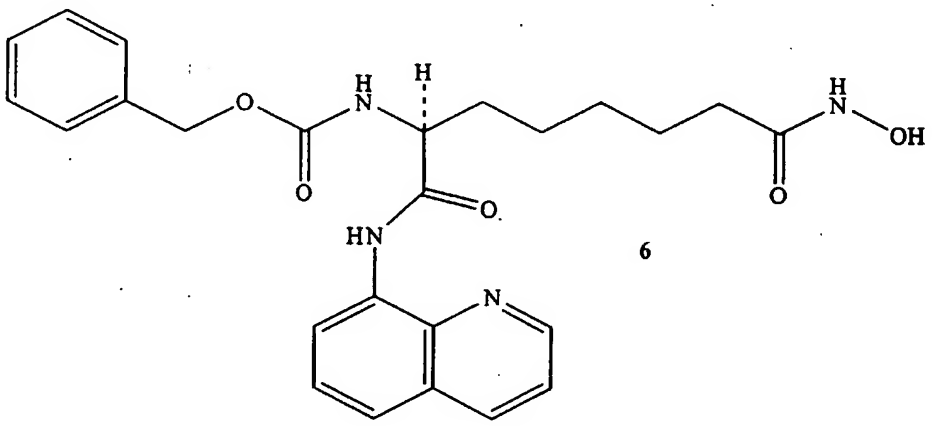
Table 1 shows a summary of the results of the Examples 7-10, testing compounds 1-4, and also compares the results to the results obtained from using SAHA.

Table 1. Summary of Test results of compounds 1-4, and comparison to SAHA results.

	MEL Differentiation			HDAC Inhibition	
Compound	Range	Opt.	%B+	Range	ID50
1	0.1 to 50 μM	200 nM	44%	0.0001 to 100μM	1 nM
2	0.2 to 12.5 μM	800 nM	27%		TBT
3	0.1 to 50 μM	400 nM	16%	0.01 to 100 μM	100 nM
4	0.01 to 50 μM	40 nM	8%	0.01 to 100 μM	<10 nM
SAHA		2500 nM	68%	0.01 to 100 μM	200 nM

Example 12 - Modified Inhibitors of HDAC

In additional studies we found that compounds **6** and **7** shown below were very effective inhibitors of the enzyme HDAC. Compound **6** had ID_{50} of 2.5 nM, and compound **7** had ID_{50} of 50 nM. This contrasts with an ID_{50} for SAHA of 1 μM, much higher. Note that the 1 μM ID_{50} for SAHA as an inhibitor of HDAC is of the same general magnitude as its 2.5 μM optimal dose for the cytodifferentiation of MEL cells, but this close similarity is not true for all the compounds examined. In some cases very effective HDAC inhibitors are less effective as cytodifferentiaters, probably because the drugs are metabolized in the cell assays. Also, all cell types are not the same, and some compounds are much better against human tumor cells such as HT-29 than they are against MEL cells. Thus, inhibition of HDAC cells is a preliminary indicator.



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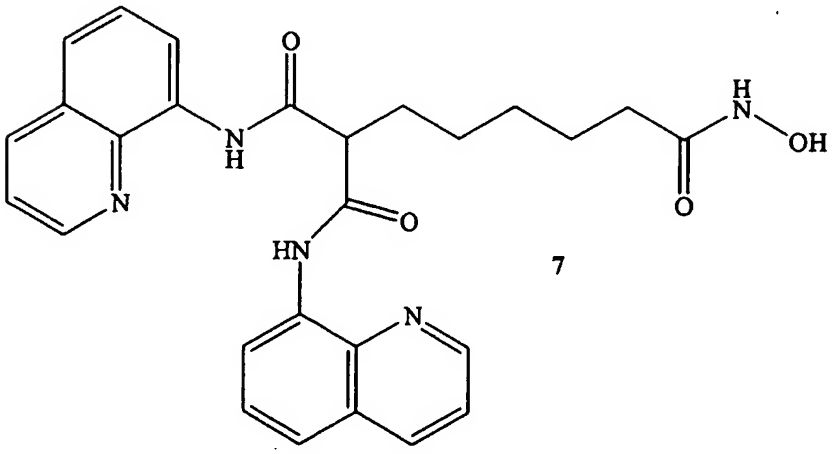

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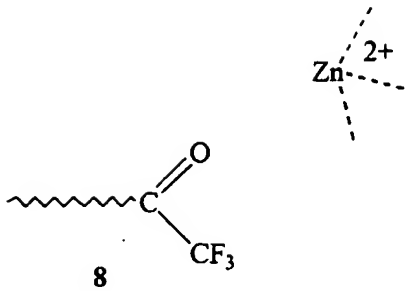
Example 13 - Evolution of Compounds without a Hydroxamic Acid Portion

Of the above compounds which are hydroxamic acids, we have found that they undergo enzymatic hydrolysis rather rapidly to the carboxylic acids, so their biological lifetimes are short. We were interested in evolving compounds which might be more stable *in vivo*. Thus we have developed inhibitors of HDAC that are not hydroxamic acids, and that can be used as cytodifferentiating agents with longer biological lifetimes. Furthermore, we found that the newly evolved compounds have better selectivity to HDAC than, e.g. SAHA.

We have evolved compounds that have double bonds, similarly to Trichostatin A (TSA) to see if the resulting compounds have even greater efficacy. Also, the chain in TSA is only five carbons, not the six of SAHA. In Oxamflatin there is a chain of four carbons containing a double bond and an ethinyl linkbetween the hydroxamic acid and the first phenyl ring, and Oxamflatin has been claimed to be an effective inhibitor of HDAC. We incorporate some of these features in our compounds, including those compounds that are not hydroxamic acids.

Also disclosed are simple combinatorial methods for screening a variety of such compounds for efficacy and selectivity with respect to HDAC inhibition.

Furthermore, since there are many important enzymes that contain Zn(II), hydroxamic acids, and perhaps some of the other metal coordinating groups, can also bind to Zn(II) and other metals.



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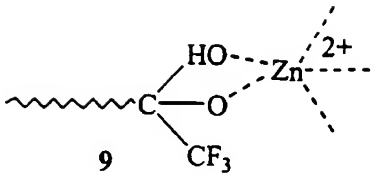

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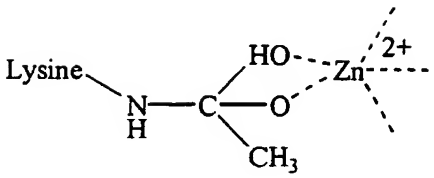

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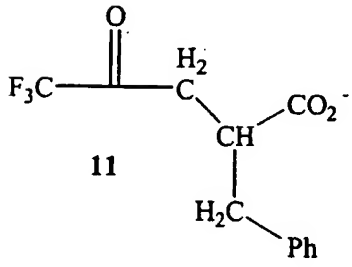

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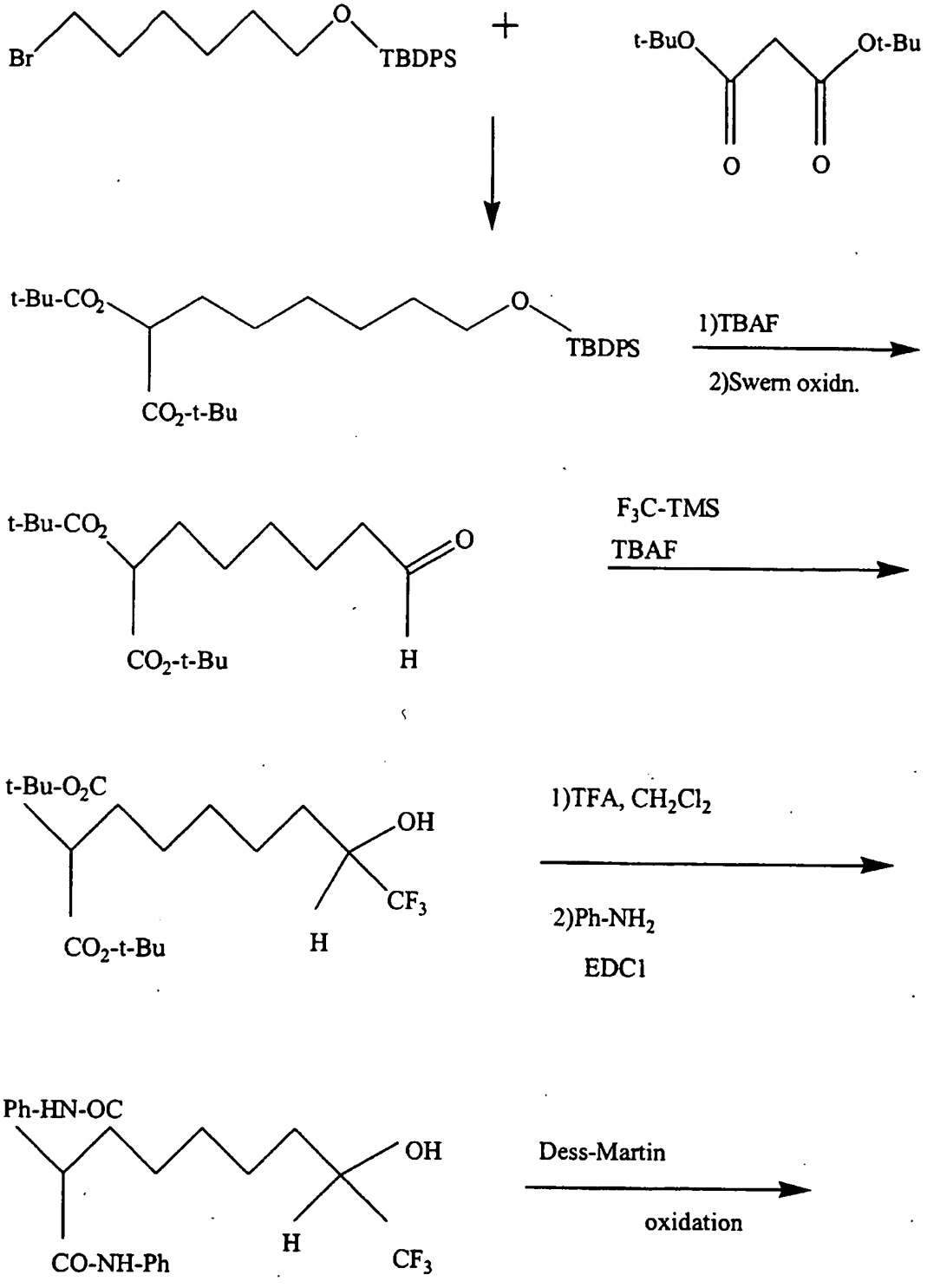
Since the target for HDAC is an acetyllysine sidechain of histone, we make compounds in which transition state analogs of the substrate are present. For example, we synthesize compounds like SAHA in which the hydroxamic acid group -CO-NHOH is replaced by a trifluoroacetyl group, $-CO-CF_3$. The resulting **8** will easily form a hydrate, and thus bind to the Zn(II) of HDAC in a mimic **9** of the transition state **10** for deacetylation. This is related to the work published by Lipscomb [56] on the binding to carboxypeptidase A of a substrate analog **11** containing a CF_3-$CO-CH_2$ group in place of the normal amide. The hydrate of the ketone coordinated to the Zn(II) as a mimic of the transition state for catalyzed hydrolysis of an amide substrate. Our synthesis of a particular example **12** in the fluoroketone series is shown in Scheme below:


Br
O
TBDPS
+
t-BuO
Ot-Bu
O
O
t-Bu-CO2
O
TBDPS
CO2-t-Bu
1)TBAF
2)Swern oxidn.
t-Bu-CO2
O
CO2-t-Bu
H
F3C-TMS
TBAF
t-Bu-O2C
OH
CF3
CO2-t-Bu
H
1)TFA, CH2Cl2
2)Ph-NH2
EDCI
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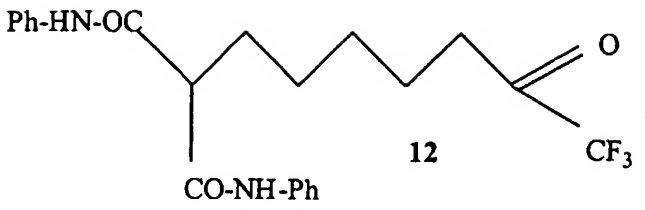
OH
H
CF3
CO-NH-Ph
Dess-Martin
oxidation

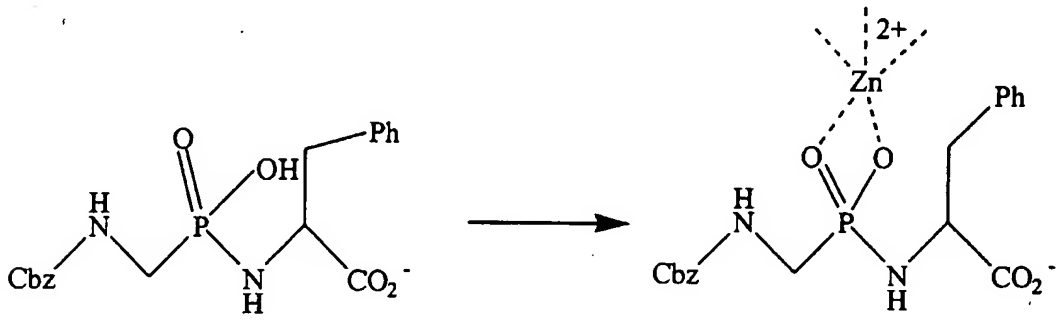


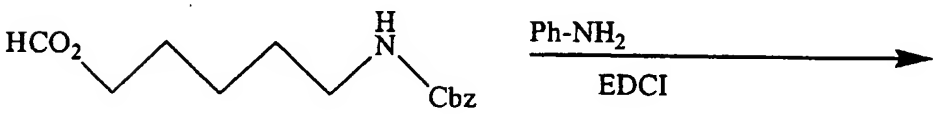
After the malonic ester alkylation, the aldehyde is prepared and then converted to the trifluoromethyl carbinol with Rupperts reagent [57, 58]. The malonic bis-anilides are prepared, and the carbinol oxidized to the ketone **12** with the Dess-Martin reagent [59]. Other approaches were tried unsuccessfully. In particular, attempts to convert a carboxylic acid derivative directly to a trifluoromethyl ketone did not work.

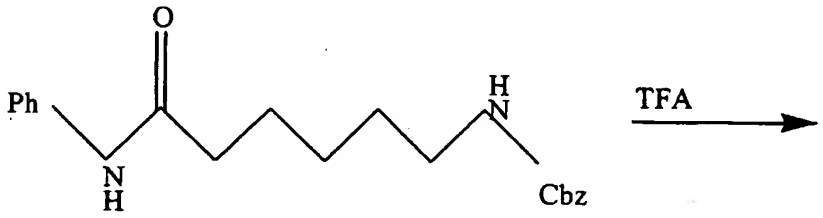
Compound **12** has been tested with HDAC and found to be an inhibitor of the enzyme. Thus, we also adapt this synthesis to the preparation of analogs of **12** with unsaturation, etc., in the chain, and other groups at the left end of the molecule.

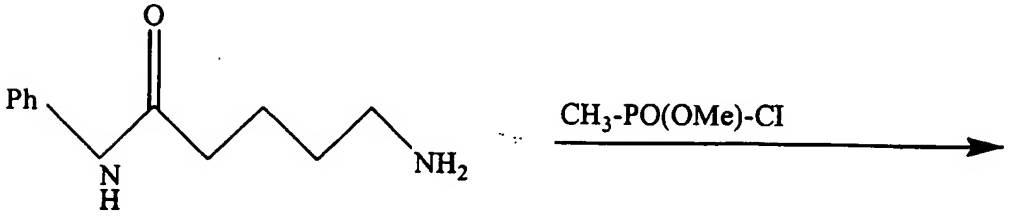
Example 14 - Evolution of Compounds where the Hydroxamic Acid Group is Replaced by NH-P(O)OH-CH_3

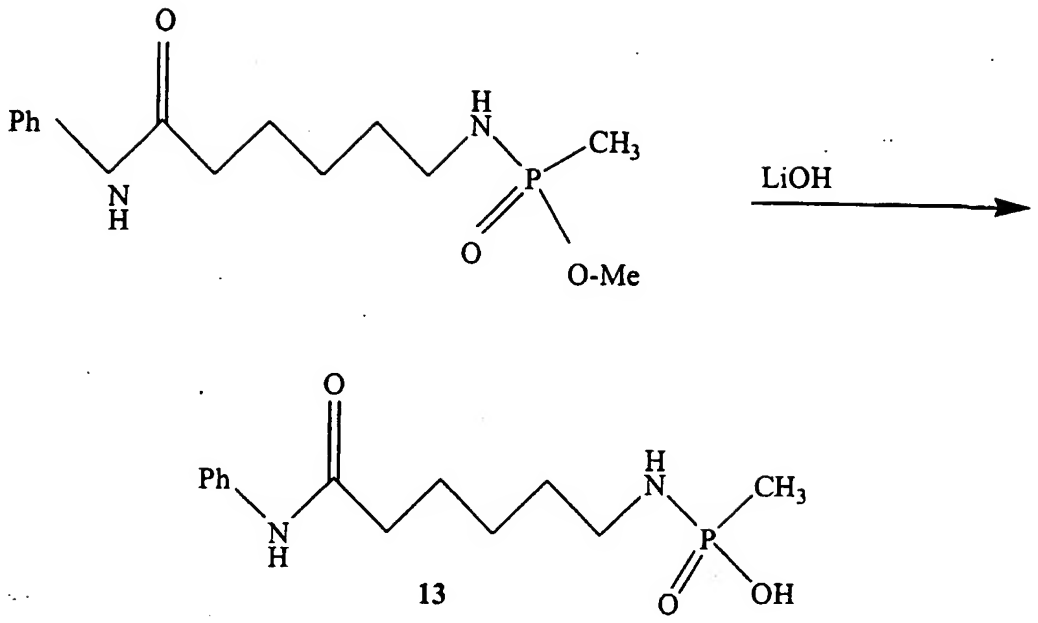
An analog of SAHA in which the CH_2-CO-NHOH group is replaced by NH-P(O)OH-CH_3 may be synthesized by the general scheme shown below. The resulting compound, **13**, binds to the Zn(II) of HDAC the way a related group binds to the Zn(II) of carboxypeptidase in analogs such as that prepared by Bartlett [60].







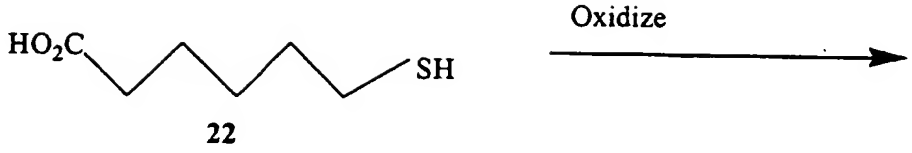




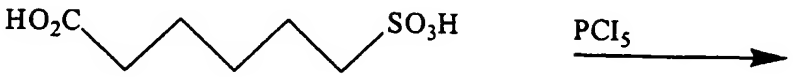
13

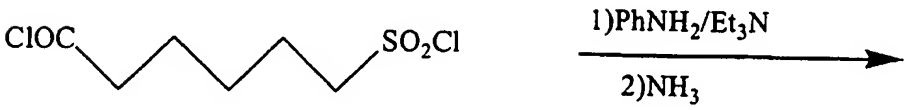
A classic inhibitor of the Zn(II) enzyme carbonic anhydrase is a sulfonamide, whose anion binds to the Zn(II) [61]. Thus compound **14**, an analog of SAHA with a sulfonamide group, is synthesized as shown below. In the last step we react a carboxylic sulfonic bis-chloride with aniline and ammonia. Since the carboxylic acid chloride reacts faster, we use the sequence of aniline, then ammonia, but the sequence may be reversed, or the mixture may be separated if the two are of similar reactivity.

In the course of the synthesis of **14**, we use a thiol **15** easily made from the corresponding haloacid. Thiols are also inhibitors of Zn(II) enzymes such as carboxypeptidase A and related peptidases such as Angiotensin Converting Enzyme (ACE), so we convert **15** to **16** as an inhibitor of HDAC. A similar synthesis can be used to attach the NH-P(O)OH-CH_3 group to other compounds, in particular compounds 6 and 7.


Oxidize
HO2C
SH

22


HO2C
SO3H
PCl5


ClOC
SO2Cl
1)PhNH2/Et3N
2)NH3


Ph-NH-CO
SO2NH2

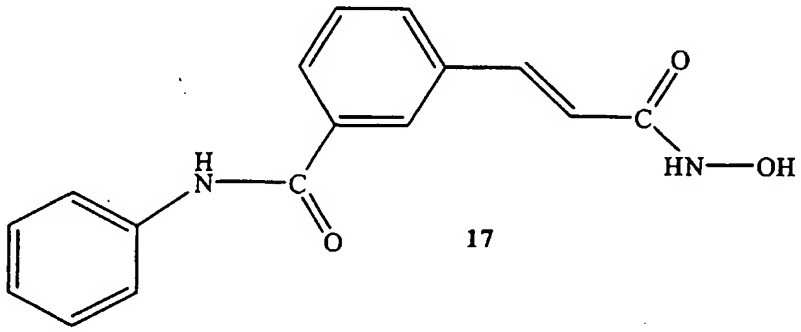
14


Ph-HN-OC
SH

16

Example 15 - Varying the linker between the Zn(II) binding group and the hydrophobic binding groups.

Based on the results with Oxamflatin, it seems that a phenyl ring can be part of the chain between the Zn(II) binding group and the left hand section of the molecule as drawn, particularly when the phenyl ring is meta substituted. Thus, we provide a synthesis to incorporate such meta substituted chains into other of our compounds. We construct compounds **17** and **18**. The simple syntheses, not shown in detail, only require that instead of the hydroxamic acid attached to the phenyl ring we make the aryl amides of **17** and **18**.



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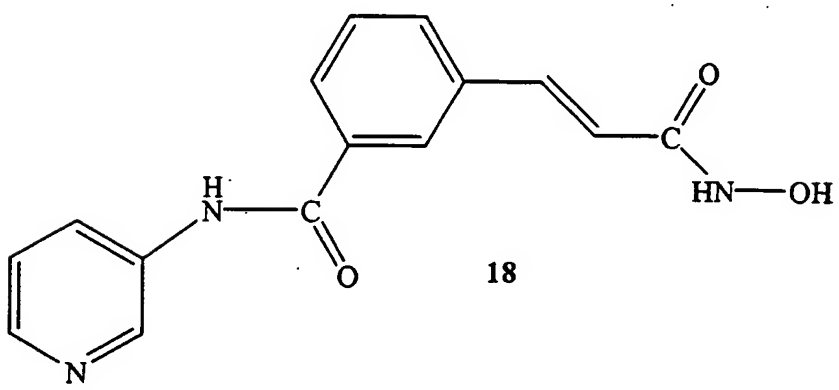
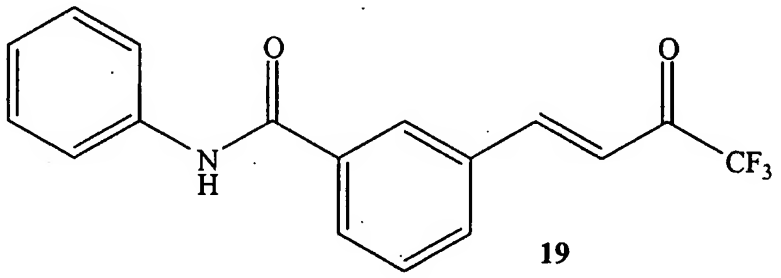


18

Additional compounds may be synthesized, such as **19** and **20** to incorporate the trifluoromethyl ketone group of **12** that we know is effective as a Zn(II) binder in HDAC. The syntheses involve preparing compounds **21** and **22** and then adding CF_3 to form the

carbinol, followed by oxidation as in the synthesis of **12**. A simple synthesis involves Heck coupling of compounds **23** and **24** with ethyl acrylate, and conversion of the ester to aldehydes **21** and **22** by reduction to the carbinol and then reoxidation.

All the chains shown so far contain only carbon atoms, but thioether links may be acceptable and even useful, and they add synthetic ease. Thus, sulfonamides such as **25** and **26**, related to **19** and **20**, from the corresponding thiophenol and bromomethylsulfonamide. A related synthesis may be used to make the corresponding phosphonamidates **27** and **28**, if this class proves to be useful HDAC inhibitors and cytodifferentiators. In this case, (N-protected) m-aminobenzoic acid is used to acylate the arylamines, then phosphorylate the anilino group.



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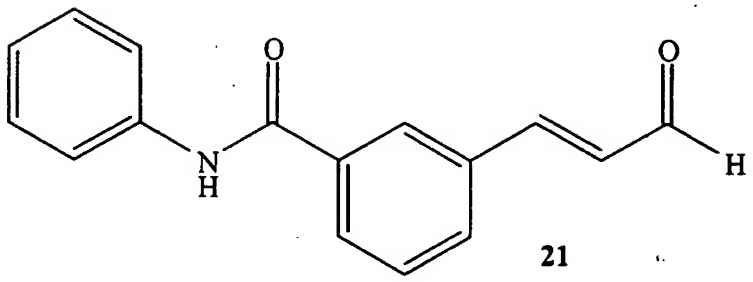

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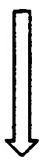

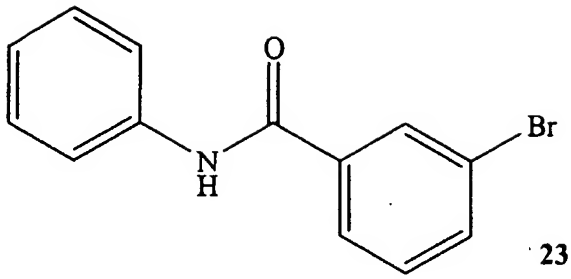

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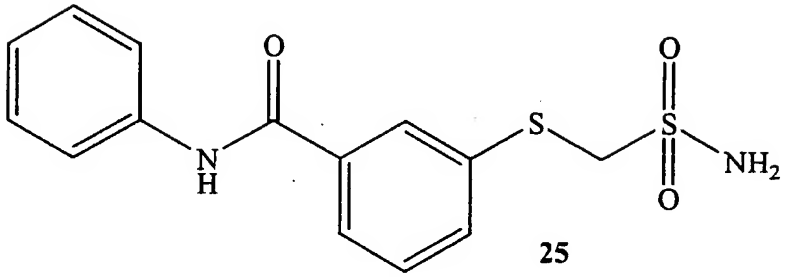

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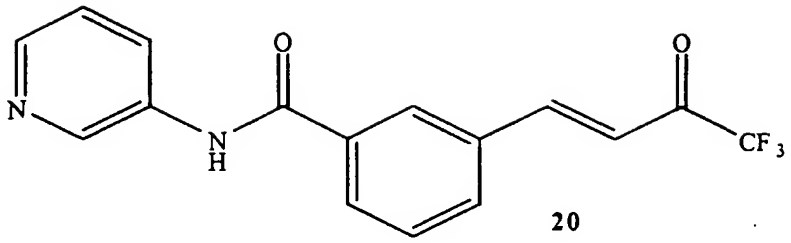

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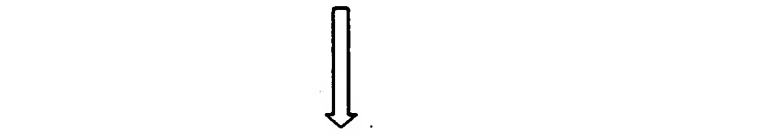

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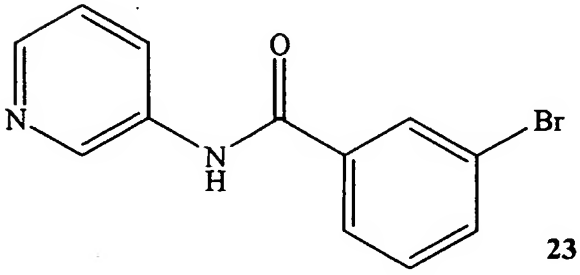

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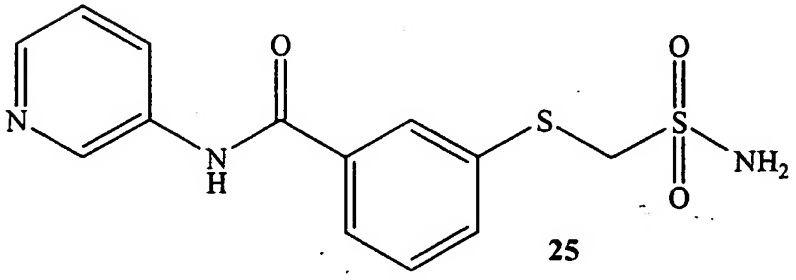

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